PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference AP102017	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/FI2004/000678	International filing date *(day/month/year)* 15.11.2004	Priority date *(day/month/year)* 18.11.2003
International Patent Classification (IPC) or national classification and IPC INV. B01L3/00 G01N1/28 F16K11/08 B01D29/66		
Applicant NURMI, Jussi		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 7 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in celectronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14.06.2005	28.03.2006
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Tiede, R Telephone No. +31 70 340-1090

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

10/579137
International application No.
PCT/FI2004/000678

IAP20 Rec'd PCT/PTO 15 MAY 2006

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-21 as originally filed

Claims, Numbers

1-17 received on 08.09.2005 with letter of 05.09.2005

Drawings, Sheets

1/8-8/8 as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 18
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-17
	No:	Claims	
Inventive step (IS)	Yes:	Claims	14-17
	No:	Claims	1-13
Industrial applicability (IA)	Yes:	Claims	1-17
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (SEPARATE SHEET)

International application No.

PCT/FI2004/000678

Re Item V.

1 The following documents are referred to in this communication:

D1 : WO 02/04921 A2 (COULTER INTERNATIONAL CORP) 17 January 2002 (2002-01-17)

D2 : US 6 106 483 A (GUIRGUIS ET AL) 22 August 2000 (2000-08-22)

D3 : US 4 581 014 A (MILLERD ET AL) 8 April 1986 (1986-04-08)

2 INDEPENDENT CLAIM 1

2.1 Document D1 discloses (the references in parentheses applying to this document): A method and system to separate and analyse particles from a sample fluid (cells) by first drawing a sample through a filter in one direction and in a further step back flushing said filter with a buffer solution into a new test tube where the particles are analysed (fig. 4; page 22, lines 15-28). Different analytical techniques may be used for the analysis of said particles (Example 8).

2.2 Document D2 discloses (the references in parentheses applying to this document): Filter and method to use said filter to separate particles from a sample fluid, comprising the step of back flushing the filtered particles in a container (i.e. fig. 11, column 8, line 32 - column 10, line 8).

2.3 Subject-matter of claim 1 differs from D1 and D2 in that the above mentioned method steps are part of a nucleic acid amplification procedure.

2.4 No surprising effect could be found in the description which go beyond those given in both document D1 and D2, namely to separate biological particles to decrease interference from unwanted substances/particles from a sample during further analysis. The choice of nucleic acid amplification must therefore be regarded as choosing a specific analysis method after a sample preparation in which back flushing a filter took place.

2.5 Both documents D1 and D2 suggest to use different analysis methods according to

the circumstances and the needs (eg. D1 page 29, lines 1-5; D2, col. 3, lines 3-18). Nucleic acid amplification is a well known technique used for analysing biological samples and it is also well known that separations have to be performed prior to the actual amplification procedures to prevent interferences, the person skilled in the art would it therefore regard it as obvious to use the biological sample separation procedures in combination with a nucleic acid amplification procedure and thus arrive at the subject-matter of claim 1.

2.6 Subject-matter of claim 1 is therefore not inventive in view of documents D1 or D2 respectively (Article 33(3) PCT).

2.7 Dependent claims 2-13 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step (Article 33(2) and (3) PCT) see citations as given above and in the International Search Report.

3 INDEPENDENT CLAIM 14

3.1 Document D1, is considered to represent the most relevant state of the art for subject-matter of claim 14. It differs from the subject-matter of claim 14 in that it does not disclose a filter integrated into a multi-way valve (see citations as given above)

3.2 The subject-matter of claim 14 is therefore novel (Article 33(2) PCT). The problem to be solved by the present invention may be regarded as: Finding a simplified arrangement to perform a separation and analysis and including back flushing a filter. This is solved by integrating the filter in a multi-way valve.

3.3 D3 does not suggest to use multi-way valves in a analysis method as disclosed in D1, nor does it disclose four ports. No incentive could be found to combine the teachings of D1 and D3. The solution to this problem proposed in claim 14 of the present application is therefore considered as involving an inventive step (Article 33(3) PCT).

3.4 Claims 15-17 comprise all technical features of claim 14. They are therefore equally

new and inventive (Article 33 (2) and (3) PCT).

Re Item VIII.

4 Present claim 14 is unclear (Article 6 PCT) for the following reasons:
Claim 14 states that "means" should be present to lead fluids through a filter, as said means are not specified also the omnipresent gravitational force would classify as means to lead a fluid through said filter. Therefore, no additional technical feature is implied by sub-paragraph c). The wording of claim 14 is therefore either not concise, or it is unclear in that it does not define the technical features necessary to obtain the desired results (liquid flow through said filter).

4.1 The same applies mutatis mutandis to the "means" referred to in claims 15 and 16.

5 Contrary to the requirements of Rule 5.1(A)(ii) PCT, the relevant background art disclosed in the documents D1-D3 is not mentioned in the description, nor are these documents identified therein.

CLAIMS

1. A nucleic acid amplification assay for quantitative and/or qualitative analysis of the presence of a specific analyte or specific analytes in a biological sample, which analytes, if present, are contained in biological particles (4) of said sample (2), in which assay the sample (2) is forced in a first direction through a filter (6) that retains said biological particles (4) **characterised** in that said biological particles (4) retained in said filter (6) are flushed, by a flush flow (8), in a second opposite direction through said filter (6) out of said filter (6) and said flush flow (8) containing said biological particles (4) flushed out is analysed for the analyte or analytes.

2. The assay of claim 1 **characterised** in that said assay comprises an additional filtration prior to the filtration retaining the biological particles (4) containing the analyte or analytes, which additional filtration does not retain the biological particles (4) containing the analyte or analytes but retains particles (10) that might interfere with the analysis of the analyte or analytes.

3. The assay of claim 1 or 2 **characterised** in that the flow containing the biological particles (4) containing the analyte or analytes flushed out is analysed for the analyte or analytes without any further purification.

4. The assay of claim 1, 2 or 3 **characterised** in that retention of the biological particles (4) containing the analyte or analytes in the filter (6) is essentially size dependent.

5. The assay of any of claims 1 to 4 **characterised** in that retention of the biological particles (4) containing the analyte or analytes in the filter (6) is essentially dependent on the chemical properties of the particle.

6. The assay of any of claims 1 to 5 **characterised** in that the biological particles (4) containing the analyte or analytes are selected from the group consisting of prokaryotic or eukaryotic cells or spores or components thereof, viruses or viral particles, complexes comprising protein and/or nucleic acid, and any combination thereof.

7. The assay of claim 6 **characterised** in that the biological particles (4) containing the analyte or analytes are selected from the group consisting of bacteria, bacterial cell, plant pollen, mithochondria, chloroplast, cell nuclei, virus, phage, chromosome and ribosome.

8. The assay of any of claims 1 to 7 **characterised** in that the means of analysing the analyte or analytes is selected from the group consisting of polymerase chain reaction (PCR), reverse transcriptase polymerase chain reaction (RT-PCR), ligase chain reaction (LCR), proximity ligation assay, nucleic acid sequence based amplification (NASBA), strand displacement amplification (SDA) and any combination thereof.

9. The assay of any of claims 1 to 8 **characterised** in that the biological particles (4) containing the analyte or analytes are flushed with a liquid or a gas preferably not contained in the original sample 2.

10. The assay of any of claims 1 to 9 **characterised** in that the analyte or analytes are selected from the group consisting of a living and/or dead cell or virus; a peptide, a protein or complex thereof; a nucleic acid; and any combination thereof.

11. The assay of claim 10 **characterised** in that the analyte or analytes comprises living and/or dead cells and/or viruses selected from the group consisting of a mold, a yeast, a eukaryotic cell or organism, a pathogenic virus and a cancer cell.

12. The assay of claim 10 **characterised** in that the analyte or analytes comprises nucleic acids selected from the group consisting of DNA, RNA and any derivative thereof.

13. The assay of claim 10 **characterised** in that the analyte or analytes comprises peptides and/or proteins or complexes thereof selected from the group consisting of a hormone, a growth factor, an enzyme or parts thereof and/or complexes thereof; and any combination thereof.

14. An arrangement (12) for preparing a biological sample (2) for quantitative and/or qualitative analysis of the presence of a specific analyte or specific analytes, which analytes, if present, are contained in biological particles (4) of the sample (2), wherein the arrangement (12) comprises

a) a housing (14) for a filter (6);

b) a filter (6) within said housing (14) for retaining the biological particles (4) containing the analyte or analytes, said filter (6) having two sides,

   i) a sample inlet side (16) and

   ii) a flushing flow inlet side (18); and

c) means for

   i) leading (20) the sample (2) through the filter (6) from the sample inlet side (16) to the flushing flow inlet side (18),

   ii) leading (22) the flush flow (8) from its inlet side (18) to the sample inlet side (16), and

   iii) retrieving (24) for analysis biological particles (4) containing the analyte flushed from the filter (6);

characterised in that the arrangement (12) comprises a filter rack (32) that is a multi-way valve, with separate connections for sample inlet (20), sample retrieval (24), flush flow inlet (36) and waste disposal (38), and optionally for wash flow (34), and the filter rack (32) with the filter (6) can be turned in alternative positions so that flow is directed from

d) the sample inlet (20) into the filter (6) from the sample inlet side (16) to the flush flow inlet side (18) and to waste (38) or optionally for use as flush flow,

e) the flush flow inlet (22) into the filter (6) from the flush flow inlet side (18) to the sample inlet side (16) and to sample retrieval (24), or

f) optionally, the flow inlet (30) into the filter (6) from the sample inlet side (16) to the flush flow inlet side (18) and to waste (38) or for recycling.

15. The arrangement (12) according to claim 14 **characterised** in that the arrangement (12) further comprises

a) an additional filter (26) that does not retain the biological particles (4) containing the analyte or analytes but retains particles (10) that might interfere with the analysis of the analyte or analytes, and

b) means for leading (28) the sample (2) through said additional filter (26) prior to leading it through the filter (6) for retaining the biological particles (4) containing the analyte or analytes.

16. The arrangement (12) according to claim 14 or 15 **characterised** in that the arrangement (12) further comprises means for leading (30) a washing liquid or gas through the filter (6) from the sample inlet side (16) to the flushing flow inlet side (18) for washing the retained biological particles (4) containing the analyte or analytes prior to flushing them out of the filter (6).

17. A kit of parts, components and/or reagents for performing the assay according to any of claims 1 to 13, **characterised** in that it comprises the arrangement (12) according to any of claims 14 to 16.

characterised in that the arrangement (12) comprises a filter rack (32) that is a multi-way valve, with separate connections for sample inlet (20), sample retrieval (24), flush flow inlet (36) and waste disposal (38), and optionally for wash flow (34), and the filter rack (32) with the filter (6) can be turned in alternative positions so that flow is directed from

d) the sample inlet (20) into the filter (6) from the sample inlet side (16) to the flush flow inlet side (18) and to waste (38) or optionally for use as flush flow,

e) the flush flow inlet (22) into the filter (6) from the flush flow inlet side (18) to the sample inlet side (16) and to sample retrieval (24), or

f) optionally, the flow inlet (30) into the filter (6) from the sample inlet side (16) to the flush flow inlet side (18) and to waste (38) or for recycling.

15. The arrangement (12) according to claim 14 **characterised** in that the arrangement (12) further comprises

a) an additional filter (26) that does not retain the biological particles (4) containing the analyte or analytes but retains particles (10) that might interfere with the analysis of the analyte or analytes, and

b) means for leading (28) the sample (2) through said additional filter (26) prior to leading it through the filter (6) for retaining the biological particles (4) containing the analyte or analytes.

16. The arrangement (12) according to claim 14 or 15 **characterised** in that the arrangement (12) further comprises means for leading (30) a washing liquid or gas through the filter (6) from the sample inlet side (16) to the flushing flow inlet side (18) for washing the retained biological particles (4) containing the analyte or analytes prior to flushing them out of the filter (6).

17. A kit of parts, components and/or reagents for performing the assay according to any of claims 1 to 13.

~~18. A kit of parts according to claim 17~~, **characterised** in that it comprises the arrangement (12) according to any of claims 14 to 16.